

Claims

1. An method for identifying and/or obtaining a compound which inhibits infectivity of a protozoan pathogen, which
5 method comprises:
 (a) contacting an isolated Rhomboid polypeptide and an isolated substrate polypeptide in the presence of a test compound; and
 (b) determining proteolytic cleavage of the substrate
10 protein.
2. A method according to claim 1 wherein the protozoan pathogen is an apicomplexan pathogen.
- 15 3. A method according to claim 2 wherein the apicomplexan pathogen is selected from the group consisting of Plasmodium, Toxoplasma, Eimeria, Sarcocystis, Cyclospora, Isospora, Cryptosporidium, Babesia and Theileria.
- 20 4. A method according to any one of the preceding claims wherein the Rhomboid polypeptide is a protozoan Rhomboid protein
5. A method according to claim 4 wherein the Rhomboid
25 polypeptide is encoded by a nucleic acid sequence shown in Table 1.
6. A method according to any one of the preceding claims wherein the substrate polypeptide comprises a luminal
30 domain and a TMD, the TMD having a region proximal to the luminal domain which comprises one or more of residues 138-144 of the Drosophila Spitz sequence (ASIASGA).

7. A method according to claim 6 wherein the substrate polypeptide comprises a TMD and a luminal domain, the TMD having a region proximal to a luminal domain which has the sequence of residues 138-144 of Drosophila Spitz.

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8. A method according to claim 6 wherein the substrate polypeptide is an adhesive micronemal polypeptide.

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9. An assay method according to claim 8 wherein the substrate polypeptide is encoded by a nucleic acid sequence shown in Table 2.

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10. An assay method according to claim 9 wherein the substrate polypeptide is Ama-1 or CTRP.

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11. A method according to any one of the preceding claims wherein the substrate polypeptide and the Rhomboid polypeptide comprise ER (endoplasmic reticulum) retention signals.

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12. A method according to claim 10 wherein the endoplasmic reticulum retention signals are KDEL or KKXX.

13. A method according to any one of the preceding claims wherein the substrate polypeptide comprises an extracellular domain having a detectable label.

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14. A method according to claim 13 wherein the detectable label is GFP.

15. A method according to any one of the preceding claims wherein said Rhomboid polypeptide and said substrate

polypeptide are expressed in a host cell from heterogeneous nucleic acid.

16. A method according to any one of the preceding claims
5 comprising the further step of;

(c) bringing into contact an isolated human Rhomboid polypeptide and a polypeptide substrate in the presence of the test compound; and,

(d) determining proteolytic cleavage of the substrate
10 by the human Rhomboid polypeptide.

17. A method according to any one of the preceding claims comprising identifying said test compound as a modulator of adhesive micronemal polypeptide cleavage.

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18. A method according to claim 17 further comprising determining the ability of said test compound to inhibit the invasiveness of a protozoan pathogen.

20 19. A method according to claim 17 or claim 18 comprising isolating said test compound.

20. A method according to claim 19 comprising synthesising the test compound.

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21. A method according to claim 19 comprising modifying the test compound to optimise its pharmacological properties.

30 22. A method according to any one of claims 17 to 21 comprising formulating said test compound in a pharmaceutical composition with a pharmaceutically acceptable excipient, vehicle or carrier.

23. A compound which modulates protozoan pathogen infectivity obtained by a method of any one of claims 1 to 18.

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24. A compound according to claim 23 comprising a peptide fragment of a protozoan Rhomboid polypeptide.

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25. A method of producing a pharmaceutical composition comprising;

identifying a compound which inhibits the infectivity of a protozoan pathogen using a method according to any one of claims 1 to 18; and,

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admixing the compound identified thereby with a pharmaceutically acceptable carrier.

26. A method according to claim 25 comprising the step of modifying the compound to optimise the pharmaceutical properties thereof.

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27. A method for preparing a pharmaceutical composition for treating a protozoan pathogen infection comprising;

i) identifying a compound which modulates the proteolytic activity of a Rhomboid polypeptide,

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ii) synthesising the identified compound, and;

iii) incorporating the compound into a pharmaceutical composition.

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28. A pharmaceutical composition comprising a compound according to claim 23 or claim 24.

29. Use of a compound according to claim 23 or claim 24 in the manufacture of a composition for treatment of a protozoan pathogen infection.

5 30. A method comprising administration of a composition according to claim 23 or claim 24 to a patient for treatment of a protozoan pathogen infection.

10 31. A method according to claim 30 wherein the protozoan pathogen is an apicomplexan pathogen selected from the group consisting of Plasmodium, Babesia, Theileria, Toxoplasma, Eimeria, Sarcocystis, Cyclospora, Isospora and Cryptosporidium.

15 32. A method of identifying a protozoan Rhomboid polypeptide comprising;
(a) providing a test protozoan Rhomboid polypeptide,
(b) bringing into contact a substrate polypeptide and the test Rhomboid polypeptide under conditions in which the
20 substrate polypeptide is normally proteolytically cleaved;
and,
(c) determining cleavage of the substrate polypeptide.

25 33. A method according to claim 32 wherein the test Rhomboid polypeptide comprises an amino acid sequence encoded by a nucleic acid sequence shown in Table 1.

30 34. A method according to claim 32 or claim 33 wherein the substrate polypeptide comprises the lumenal region of the TMD of Spitz, Gurken, Keren, Ama-1 or CTRP.

35. A method according to any one of claims 32 to 34 wherein the substrate polypeptide comprises an amino acid

sequence encoded by a nucleic acid sequence shown in Table 2.

36. A method according to claim 35 wherein the substrate
5 polypeptide is Ama-1 or CTRP.